REMARKS

A. Status of the Claims

Claims 25-27 are pending and stand rejected.

B. Reference AF

Applicants have included herewith a copy of reference AF for the Examiner's consideration.

C. Priority Information

Applicants note that the priority information was updated in the Preliminary Amendment filed November 4, 2003. Acknowledgment of the amendment to the specification is respectfully requested.

D. Rejection under 35 U.S.C. §103(a)

Claims 25-27 stand rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Nichols et al., U.S. Patent Number 5,099,005 in view of Zuk et al., U.S. Patent Number 4,281,061. Applicants respectfully disagree because:

(1) Neither Nichols nor Zuk teach the use of an N-glycosidase or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid to produce $F(ab')_2$ fragments;

(2) Nichols ***teaches away*** from the use of an N-glycosidase and an O-glycosylase; and

(3) None of the cited references provide a suggestion to modify the methods of Nichols to employ an N-glycosidase and/or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid for the purpose of producing $F(ab')_2$ fragments.

1. Burden of Procedure in Establishing Prima Facie Obviousness

"The examiner bears the burden of establishing a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993); *In re Oetiker*, 977

F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Only if this burden is met does the burden of coming forward with rebuttal arguments or evidence shift to the applicant. *Rijckaert*, 9 F.3d at 1532, 28 USPQ2d at 1956. When the references cited by the examiner fail to establish a *prima facie* case of obviousness, the rejection is improper and will be overturned. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988)." See *In re Deuel*, 51 F.3d 1552, 34 USPQ2d 1210, 1214 (Fed. Cir. 1995).

In order to establish a *prima facie* case of obviousness, the rejection must demonstrate that (1) the cited references teach all the claimed elements; (2) there is a suggestion or motivation in the prior art to modify or combine the reference teachings; and (3) there is a reasonable expectation of success. MPEP § 2143; *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). As explained below, the cited references fail to disclose all the elements of the claimed invention, do not provide a suggestion or motivation to combine the references, and fail to provide a basis for one of skill to reasonably expect that Applicants' kit would be useful in producing $F(ab')_2$ fragments.

2. The Art of Record Fails to Teach Each Claim Element

Claim 25 encompasses a kit for making $F(ab')_2$ fragments from a glycosylated antibody. The kit of claim 25 includes "a deglycosylation composition comprising at least one glycosidase capable of catalyzing the hydrolysis of an N-glycosidic or O-glycosidic linkage between a sugar unit and an amino acid to form a partially or wholly deglycosylated antibody." Therefore, to meet all the elements of claim 25, the cited combination of references must minimally teach the treatment of a glycosylated antibody with an N-glycosidase and/or an O-glycosylase capable of cleaving an antibody glycan at the amino acid to which the glycan is attached for the purpose of producing $F(ab')_2$ fragments.

Nichols fails to teach the use of an N-glycosidase and/or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid to produce $F(ab')_2$ fragments. Rather, Nichols specifically teaches that antibodies should be treated with a ***sialidase only*** to remove the terminal sialyl residues of the antibody oligosaccharides. The specification repeatedly refers to the desialylation step and the production of desialylated

antibodies as a necessary component of the invention and not merely an embodiment of the invention. *See* column 2, lines 40-52; column 5, lines 26-28; and Abstract.

It is well known in the art that sialidases (also referred to as neuraminidases) specifically hydrolyze the glycosidic bond between a sialic acid and a sugar unit, and not between a sialic acid and an amino acid. *See* JM Lackie & JAT Dow (1999) "The Dictionary of Cell & Molecular Biology" (Third edition), Academic Press, London. (ISBN 0-12-432565-3); *see also* http://www.biochem.ucl.ac.uk/bsm/enzymes/ec3/ec02/ec01/ec0129/ describing functional activity of endo-alpha sialidase. Thus, a sialidase is not "capable of catalyzing the hydrolysis of an N-glycosidic or O-glycosidic linkage between a sugar unit and an amino acid to form a partially or wholly deglycosylated antibody " as recited in claim 25.

Because neither Nichols nor Zuk describe production of $F(ab')_2$ fragments using a deglycosylation composition comprising "at least one glycosidase capable of catalyzing the hydrolysis of an N-glycosidic or O-glycosidic linkage between a sugar unit and an amino acid," a proper *prima facie* case of obviousness cannot be set forth. Therefore, Applicants respectfully request withdraw of the rejection under 35 U.S.C. § 103.

3. There is No Reasonable Expectation of Success: Nichols Teaches Away from the Claimed Invention

Applicants respectfully assert that the cited references provide no reasonable expectation of successfully using an N-glycosidase and/or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid for the purpose of producing $F(ab')_2$ fragments. In fact, Nichols ***teaches away*** from the use of glycosidases other than sialidases.

Nichols teaches that ***only a sialidase*** is capable of enhancing the yield of $F(ab')_2$ fragments. At column 6, lines 27-45, Nichols explain the basis for the yield enhancement of $F(ab')_2$ fragments after treatment with a sialidase:

> After desialylation of intact immunoglobulin, however, the desialylated immunoglobulin has heavy chains exhibiting the same or substantially similar molecular weight, and therefore both heavy chains and both light chains of the immunoglobulin will be exposed to the proteolytic enzyme affecting the

> fragmentation reaction to the same extent. As a result, the yield of the desired fragment is expected to increase.

Thus, Nichols teach that the key to $F(ab')_2$ yield enhancement is the production of immunoglobulin heavy chains exhibiting substantially the same molecular weight. At column 5, lines 26-34, Nichols explicitly teach that ***only desialylation and not deglycosylation*** produces heavy chains of the same molecular weight:

> This yield enhancement method exploits the observation that the differences in immunoglobulin heavy chain molecular weight are attributable to ***asymmetric sialylation*** of the heavy chains, ***rather than asymmetric glycosylation*** thereof.

Here, Nichols state that asymmetric glycosylation is not the cause of differences in the molecular weights of the heavy chains. Thus, after examining the above passages, one of skill would necessarily conclude that the use of a deglycosylating enzyme other than a sialidase would produce heavy chains of substantially different molecular weight leading to a difference in heavy chain protease fragmentation, and ultimately resulting in poor $F(ab')_2$ production. Therefore, Nichols teaches away from the use of a deglycosylation composition comprising "at least one glycosidase capable of catalyzing the hydrolysis of an N-glycosidic or O-glycosidic linkage between a sugar unit and an amino acid."

Because Nichols teaches away from the use of glycosidases other than sialidases, the cited references provide no reasonable expectation of successfully producing $F(ab')_2$ fragments using an N-glycosidase and/or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid.

4. There is No Suggestion to Modify the Reference Teachings

At present, there are no references of record that either expressly or impliedly contain a suggestion to modify the methods of Nichols to employ an N-glycosidase and/or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid to produce $F(ab')_2$ fragments as recited in the claims. Nichols does not suggest that there is any deficiency in the disclosed methods that could be remedied by replacing or combining a sialidase with an N-glycosidase and/or an O-glycosylase capable of hydrolyzing a glycosidic linkage

between a sugar unit and an amino acid to produce $F(ab')_2$ fragments. The only suggestion to use an N-glycosidase and/or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid to produce $F(ab')_2$ fragments is found within Applicants' own specification.

Therefore, Applicants respectfully submit the there is no suggestion to modify the method of Nichols to obtain a kit for producing $F(ab')_2$ fragments containing an N-glycosidase or an O-glycosylase capable of hydrolyzing a glycosidic linkage between a sugar unit and an amino acid.

E. Double Patenting Rejection

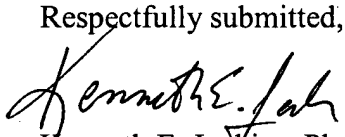
Claims 25-27 stand rejected under the judicially created doctrine of double patenting over claims 1-9 of U.S. Patent No. 6,720,165. Applicants request that this rejection be held in abeyance until allowable subject matter is found, at which point appropriate action will be taken to obviate the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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APPLICANT

Table of Contents

CALBIOCHEM®

Catalog 1994/1995

PRODUCT	CAT. NO.	UNIT SIZE	QTY	UNIT PRICE

NEURAMINIDASE

Neuraminidase, *Arthrobacter ureafaciens*

480714 | 1 U | 1-4 $ 110.00; 5+ 95.00 | ★

Solid lyophilized from 10 mM phosphate buffer, pH 7.0. Preferentially hydrolyzes terminal α-2,6-linkages between N-acetylneuraminic acid and 2-acetamido-2-deoxy-D-galactose residues in various mucopolysaccharides. Activity is independent of Ca^{2+} and is not inhibited by EDTA. In the presence of detergent (e.g., sodium cholate or TRITON® X-100), can hydrolyze N-acetylneuraminyl moiety of monosialoganglioside GM_1, that was thought to be resistant to various neuraminidases of viral, bacterial and mammalian origin. Prepared from nonpathogenic bacteria. *Specific activity: 75 units/mg protein. One unit is defined as the amount of enzyme that will liberate 1.0 µmol of N-acetylneuraminic acid (NANA) from NAN-lactose per minute at 37°C, pH 5.0. Contaminants: aldolases, glycosidases, NANA and proteases: nil.* EC 3.2.1.18, pH optimum 5.0-5.5 (NANA-lactose as a substrate), 4.5-7.0 (bovine submaxillary mucin as a substrate) and 4.3-4.5 (colominic acid as a substrate), RTECS QQ3450000, CAS 9001-67-6, M.W. 89,000 and 51,000.

Ref.: Fukudome, K., et al. 1989. *Virology* **172**, 196; Vlasak, R., et al. 1988. *Proc. Natl. Acad. Sci. USA* **85**, 4526; Corfield, P.A., et al. 1983. *Biochim. Biophys. Acta* **744**, 121; Saito, M., et al. 1979. *J. Biol. Chem.* **86**, 1573.

Neuraminidase, Isozyme S, *Arthrobacter ureafaciens*

480719 | 1 U | 1+ $ 95.00 | ✪

Lyophilized solid. Hydrolyzes N-acetylneuraminyl moiety of polysialogangliosides to produce asialoganglioside GA_1 in the presence of detergents and monosialoganglioside GM_1 in the absence of detergents and Ca^{2+}. *Specific activity: 80 units/mg protein. One unit is defined as the amount of enzyme that will liberate 1.0 µmol of N-acetylneuraminic acid (NANA) from NAN-lactose per minute at 37°C, pH 5.0.* EC 3.2.1.18, RTECS QQ3450000, CAS 9001-67-6, M.W. 52,000.

Ref.: Ohta, Y., et al. 1989. *J. Biochem.* **106**, 1086.

Neuraminidase, *Clostridium perfringens*

480715 | 1 mg | 1-4 $ 50.00; 5+ 45.00 | ✪

Brown solid. *Activity: 4.0 units/mg dry weight. One unit is defined as the amount of enzyme that will liberate 1.0 µmol of N-acetylneuraminic acid (NANA) from NAN-lactose per minute at 37°C, pH 4.5.* EC 3.2.1.18, RTECS QQ3450000, CAS 9001-67-6.

Neuraminidase, *Vibrio cholerae*

(Acylneuramunyl hydrolase)

480717 | 1 U | 1-4 $ 45.00; 5+ 40.00 | ★ ❖ | BULK!

1.0 unit/ml in 4 mM $CaCl_2$, 50 mM NaOAc, 154 mM NaCl, pH 5.5; preservative-free. Hydrolyzes O-ketosidic α-2,3-, α-2,4-, α-2,6- and α-2,8-linkages of terminal N-acetylneuraminic acid in various oligosaccharides, polysaccharides, mucopolysaccharides, glycoproteins and gangliosides. *Activity: 1.0 unit/ml. One unit is defined as the amount of enzyme that will release 1.0 µmol of N-acetylneuraminic acid (NANA) from human acid α_1-glycoprotein per minute at 37°C in 50 mM NaOAc, pH 5.5, containing 1 mM $CaCl_2$, and 1.75 g/L POLYGELINE™ Modified Collagen (Cat. No. 372400).* EC 3.2.1.18, RTECS QQ3450000, CAS 9001-67-6, M.W. 90,000.

Ref.: Galen, J.E., et al. 1992. *Infect. Immun.* **60**, 406; Tomlinson, S., et al. 1992. *Glycobiology* **2**, 549.

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